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Identification of a New Candidate Mutation, G1629R, in a Family With Type 2A von Willebrand Disease

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Type 2A is a qualitative variant of von Willebrand disease (vWD) characterized by a reduced platelet-dependent function, associated with an absence of large multimers. A G5135A transition, resulting in a glycine to arginine substitution at the codon 1629 of the von Willebrand factor, was identified by automated sequencing in one type 2A vWD Spanish patient. To detect this new candidate mutation a modified primer that creates a *Dde*I restriction site when the mutation is present was designed. This approach allowed detection of the mutation in the other three patients from the same family. On the other hand, the fact that this new mutation was not found in the 110 normal alleles screened further supports their causal relationship with the disease. Am. J. Hematol. 60:309–310, 1999. © 1999 Wiley-Liss, Inc.

Key words: von Willebrand disease; von Willebrand factor; type 2A vWD

BRIEF REPORT

von Willebrand Disease (vWD) is the most frequently inherited bleeding disorder in humans, and is caused by a qualitative and/or quantitative abnormality of the von Willebrand factor (vWF). Type 2A (vWD) refers to qualitative variants with reduced interaction between the vWF and platelets, and a lack of the high molecular weight (HMW) multimers [1]. Many of the mutations associated with this vWD form have been localized in the A2 domain of vWF, which is encoded by the 3′ region of exon 28 [2] (vWF data base: http://mmg2.im.med.umich.edu/vWF/).

Four patients from a Spanish family who bled excessively after surgery and dental extractions were classified as type 2A vWD on the basis of the laboratory data. All of them showed a lack of HMW multimers, a decrease in the medium molecular weight multimers, and an increase in the intensity of the flanking bands in the triplet structure of plasma multimers. The ristocetin-induced platelet agglutination (RIPA) was low in the four patients. The propositus showed (mean of three or more determinations): FVIII:C, (41 ± 23) U/dl; vWF:Ag, (31 ± 14) U/dl; vWF:RCo, (4 ± 1) U/dl. The patient's vWF:RCo was increased to 46 U/dl 1 hr after the infusion of the DDAVP (0.3 μg/kg body weight). The other three patients—brother, mother, and uncle—showed a fairly similar laboratory data pattern.

A 770 bp fragment from propositus genomic DNA was amplified using primers F(5′-AAGCAGGCCCCT-GAGAACAA-3′) and R(5′-ATACCAGGTGCAGGG-GAGAG-3′), corresponding to nucleotides 8229-8998 [3] in exon 28 of the vWF gene. The mismatches between gene and pseudogene are underlined. The fragment was specific for the gene as checked by *Nco*I cleavage. After purification of DNA, the transition G5135A was identified as a heterozygous mutation by automatic sequencing. This produces the G1629R amino acid substitution in the pre-pro vWF (G866R of the mature subunit). This change has not been described previously. Straightforward detection of this mutation was carried out by restriction analysis of a 202 bp fragment obtained with R and C primers (C:5′-CTGCCTGGAGACATC-CAGGTGGTGCCC**c**TT-3′, where a modified nucleotide is written in bold small letter). When the mutation is present, the primer C creates a *Dde*I restriction site that produced two bands of 174 bp and 28 bp, as can be seen in Figure 1. This allowed us to identify the transition G5135A in the four patients. In addition, this kind of

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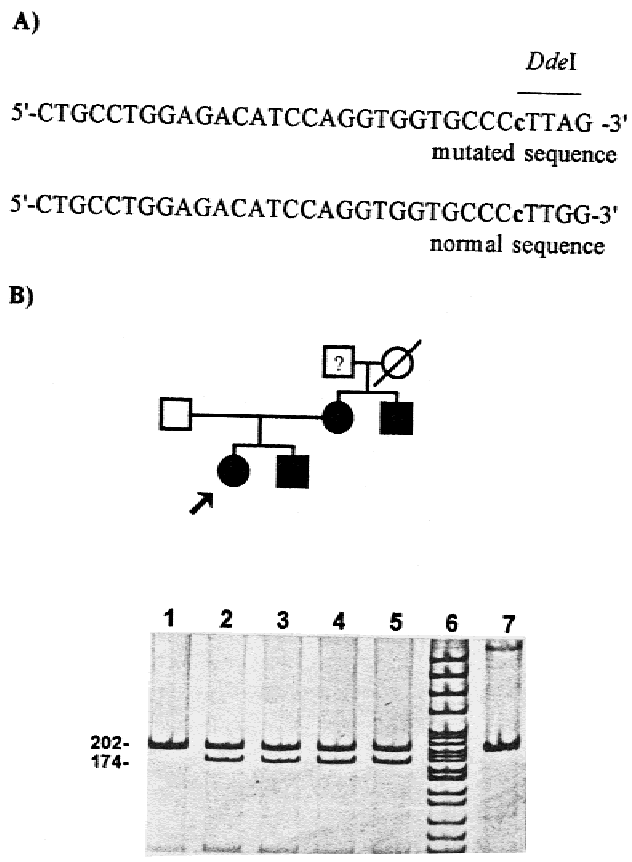

Fig. 1. A: Modified primer C, the modified nucleotide is written in bold small letter. It creates a *Dde*I restriction site when the G5135A mutation is present. B: Squares represent males, circles symbolize females, filled symbols are affected members, the arrow indicates the propositus, and slashed symbols denote deceased individuals. Electrophoresis 10% polyacrylamide, 202 bp fragment amplified with primers C and R and after *Dde*I digestion. The mutated alleles produce 174 bp and 28 bp bands (the small 28 bp band was not present in the gel). Lane 6: DNA marker (pBR322 *Msp*I digest), and lane 7: nondigested sample.

analysis was performed in 110 unrelated normal alleles, and the mutation was not detected. These results suggest that the G1629R mutation could be responsible for type 2A vWD, although only mutant expression studies will provide direct evidence of this.

Other missense mutations have been reported in the A2 domain of the vWF (vWF database). The pathophysiological mechanism by which G1629R mutation could give rise to type 2A vWD is not known. The response to DDAVP and the normal platelet vWF detected in two of the patients suggest a group 2 mechanism, where the abnormal conformation could produce an increase of susceptibility of vWF to plasma proteases after secretion into plasma [4–6]. Moreover, it is striking that mutations in codons encoding for glycine were significantly more frequent in type 2A vWD than in the other qualitative variants. Taking the above mentioned database as an unbiased reference, five of the 43 missense mutations described for type 2A are G→R changes, and two others are G→E substitutions. By comparison, no G→R change is seen in the rest of the qualitative variants, and only one type 2M case corresponds to substitution in a glycine ($P \leq 0.01$, by the Fisher's exact test). Moreover, the glycine amino acid at codon 1629 is conserved in the porcine sequence vWF, specifically in a segment between S1593 and P1632 that is 95% conserved [7]. The high degree of homology suggests that a strict conformation may be required in this area for correct protein function or processing, and the glycine might play an important role in maintaining this conformation.

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